

REPORT DOCUMENTATION PAGE

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14. ABSTRACT Our goal is to form a polygonal nanospring by self-assembly, from a designed strut-like chimeric protein. This protein is made from fragments of the T4 tail fiber and binds specifically to its angle protein. We have designed and expressed this chimeric protein which will inhibit the burst size of a T4 infected bacterium. This indicates that the chimeric protein can fold and oligomerize to create a site that binds tightly enough to the angle protein to sequester it and thereby greatly reduce the phage burst size.					
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5a. CONTRACT NUMBER. enter all contract numbers as they appear in the report, e.g. F33615-86-C-5169.

5b. GRANT NUMBER. enter all grant numbers as they appear in the report, e.g. AFOSR-82-1234.

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FINAL REPORT

GRANT #: N00014-98-1-0784

PRINCIPAL INVESTIGATOR: Edward Goldberg

INSTITUTION: Tufts University School of Medicine

GRANT TITLE: Structure of the Phage T4 Tail Fiber Angle

AWARD PERIOD: April 1, 1999 – September 30, 2000

OBJECTIVE: To demonstrate self-assembly of a non-trivial structure from a genetically engineered strut-like chimeric protein and an angle protein.

APPROACH: The proteins are derived from T4 tail fiber and redesigned to form a chimeric rod (36~34). When the chimeric rod is mixed with the angle protein, they should combine to form an intermediate which will polymerize by self assembly to form octagonal helices. We call the helix a nanospring.

ACCOMPLISHMENTS:

1. We made three chimeras of the N-terminal segment of gene 36 and the C-terminal segment of gene 34 (which contain the distal and proximal domains, respectively), that bind to the angle protein, gp35. In order to test functionality of each of these strut-like (36~34) chimeras, we expressed the clone of the putative chimeric strut and then infected the cell with wild type T4 DNA. We found that one of them, 3A (the longest), was able to inhibit normal tail fiber assembly in the infected bacteria. This suggests that the recombinant chimeric strut assembled sufficiently to form appropriate binding site(s) to the gp35 angle protein, thereby sequestering gp35 and reducing normal phage production.
2. We constructed an expression plasmid containing all three genes required for assembly of nanosprings, i.e. 57, 36~34 and 35. We induced cells for 15' and then added rifampicin for 20' to

allow degradation of most of the cellular mRNA. Subsequent labeling of protein with ^{35}S -Met put most of the label into proteins induced from the plasmid. We compared protein production (of boiled extracts) of an optimized production strain expressing gp(36~34) and P57 with an unoptimized strain producing gp(36~34), P57 and gp35. After SDS-PAGE we found copious amounts of the gp(36~34) and of gp57 in the optimized strain, but lesser amounts of these proteins in the unoptimized strain which also had little or no gp35. We will optimize gp35 expression in this context (by screening after selection for growth on IPTG plates) in order to permit good assembly of the parts.

CONCLUSIONS: We have demonstrated that a (36-34) chimera can be constructed that retains structural and functional integrity. This is illustrated by reduced formation of infective phage from the infected cell. Our hypothesis is that the chimera binds the angle protein strongly enough to prevent the normal 36 from attaching, thereby reducing the concentration of free 35 protein from participating in normal phage morphogenesis.

SIGNIFICANCE: Further work should enable us to express more of the 35 protein and thus to permit assembly in vivo to accomplish our stated goal. Such a demonstration would expand the utility of this methodology for use in the practical design and manufacture of nanoarchitectures and advance the ultimate goal of rationally designed deterministic nanoarrays and devices.

PATENT INFORMATION: None

AWARD INFORMATION: None

PUBLICATIONS AND ABSTRACTS: None

PUBLICATIONS: Hyman, P, R. Valluzzi and E.B. Goldberg (2002) Design of Protein Struts for Self-Assembling Nanoconstructs. Proc. Nat'l. Acad. Science (in the press).

Summary

Our goal is to form a polygonal nanospring by self-assembly, from a designed strut-like chimeric protein. This protein is made from fragments of the T4 tail fiber and binds specifically to its angle protein. We have designed and expressed this chimeric protein which will inhibit the burst size of a T4 infected bacterium. This indicates that the chimeric protein can fold and oligomerize to create a site that binds tightly enough to the angle protein to sequester it and thereby greatly reduce the phage burst size.

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